Pilot scale acetone, butanol, and ethanol fermentation using wastewater algae

Joshua T. Ellis, Yessica A. Castro, Reese Thompson, Charles D. Miller, and Ronald C. Sims

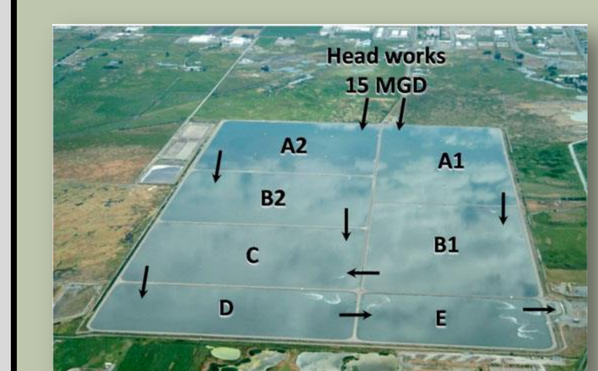
Utah State University, College of Engineering

Abstract

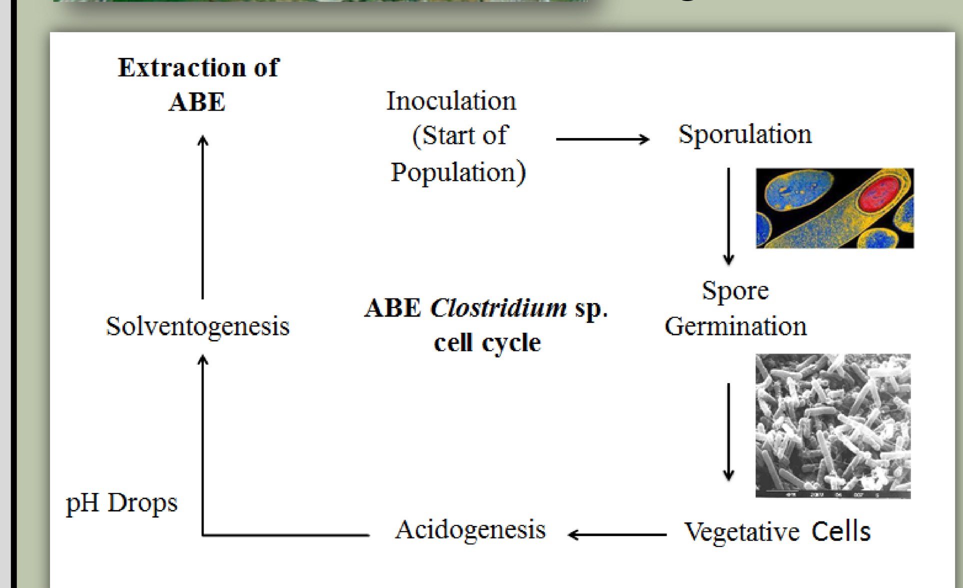
The ability to engineer novel systems for the production of high value bioproducts such as acetone, butanol, and ethanol (ABE) from renewable algae feedstocks has been demonstrated. ABE is produced by anaerobic and solventogenic clostridia, where ABE is typically produced in a 3:6:1 ratio respectively. These microorganisms initially ferment reduced sugars, producing acids, namely acetic and butyric acid, as metabolic byproducts. Solventogenesis occurs once acids reach a critical point, in which case these acids are assimilated into the cell, reduced back to their respective CoA intermediates, and further reduced to produce ABE. Batch fermentations utilizing *Clostridium saccharoperbutylacetonicum* have been shown to produce ABE from wastewater microalgae from the Logan City Wastewater Lagoon system. This algae grow naturally at high rates providing an abundant source of renewable algal biomass. Additionally, ABE has been produced using feedstocks such as glucose, xylose, lactose, and cheese whey at the laboratory scale. These data at the laboratory scale are vital for establishing procedures and understanding the physiology of the system prior to scale up efforts.

ABE production has been demonstrated using a 110L pilot scale bioreactor with glucose as feedstock. This validates proper functionality of our engineered bioreactor and provides proof of concept at the pilot scale. We are currently working on producing ABE from wastewater microalgae using the 110L bioreactor. The capacity to efficiently produce these high value bioproducts from wastewater microalgae at the pilot scale establishes a novel method for stimulating sustainable and domestic energy economies.

ABE Production and Scale Up

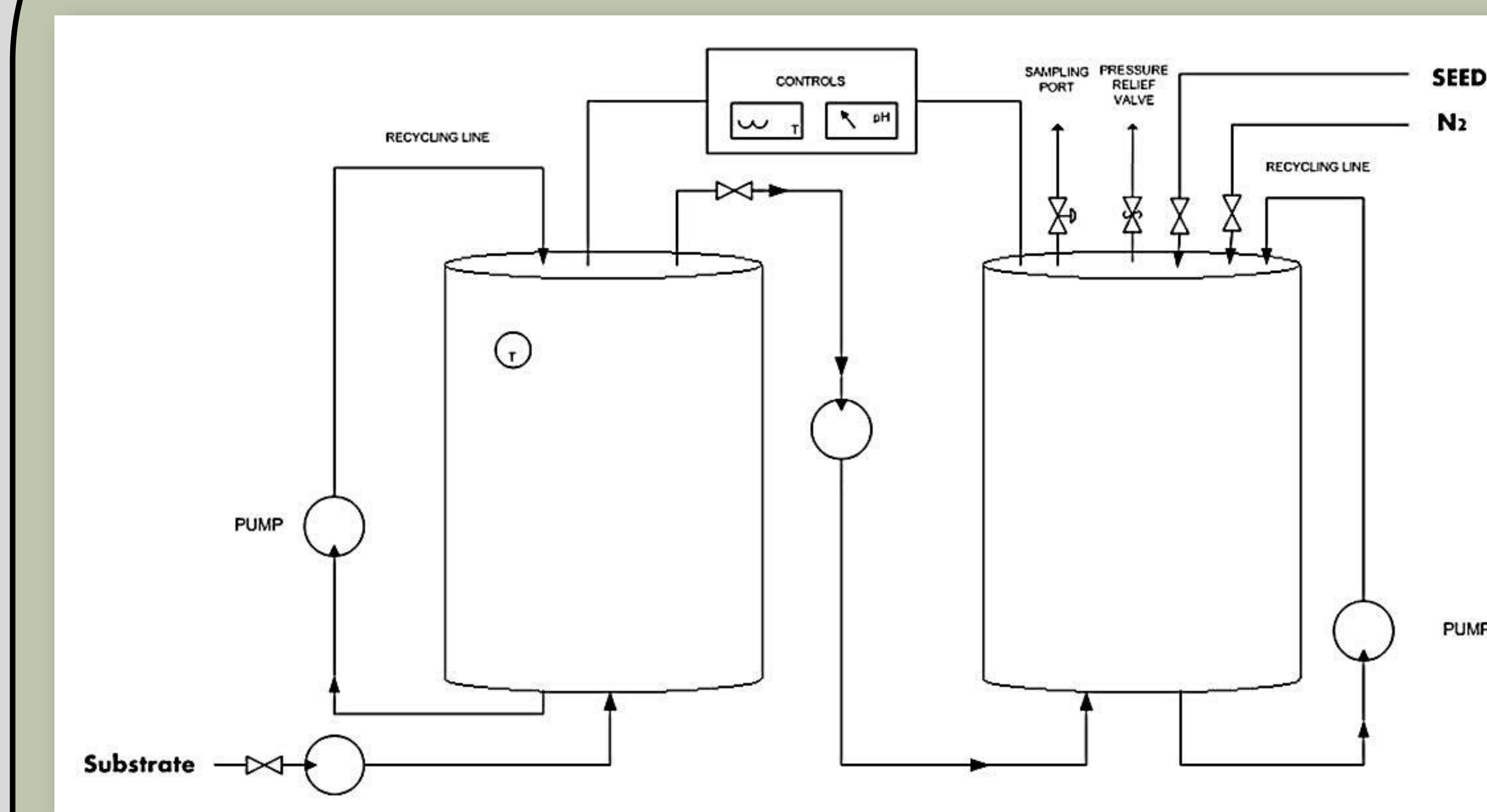


Logan, Utah treats municipal wastewater using this 460 acre Lagoon system. This system naturally facilitates algae growth which is used as feedstock to produce high value bioproducts, namely acetone, butanol, biodiesel, and bioplastics. These products are all high value products that are produced using the integrated process shown in the diagram below.



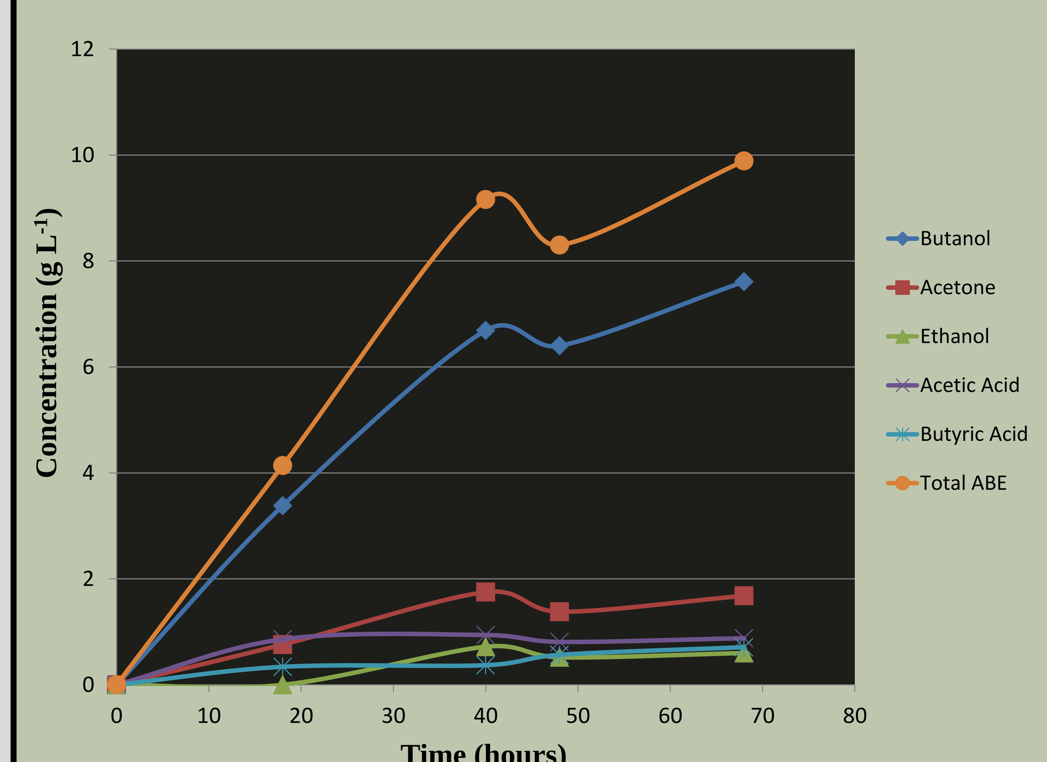
Schematic depicting the process of clostridia proliferation and ABE production

A pair of modified 110L bioreactors for pilot scale ABE production from algae biomass. A) Pretreatment reactor to lyse algae cells for increased ABE yields. B) The ABE bioreactor for producing acetone, butanol, and ethanol from algae.



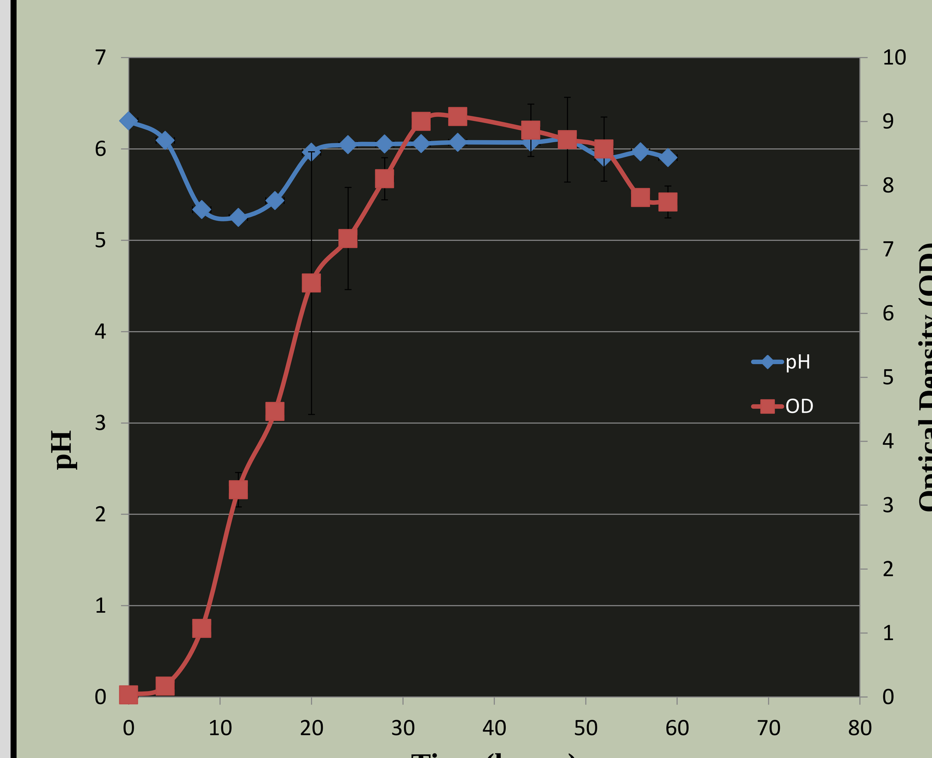
ABE anaerobic reactor system schematic. Substrate is fed into the pretreatment tank and heated at 90C for 30 minutes along with agitation and acid/base supplementation. Once pretreatment is finalized, the substrate (algae) is pumped into the ABE reactor (on the right). Media, a 5% seed culture, and N_2 are introduced to initiate ABE production and maintain anaerobic conditions. A recycle line is present on the ABE reactor to mix the culture.

Results: Pilot Scale



ABE and acid production using the 110L bioreactor from *C. saccharoperbutylacetonicum* using 3% glucose. This figure demonstrates the functionality our engineered anaerobic bioreactor. A total of 9.89 g/L of overall ABE was produced at 68 hours of fermentation, with 7.61 g/L butanol, 1.68 g/L acetone, and 0.60 g/L ethanol.

Results: Laboratory Scale



pH and optical density (OD) values from *C. saccharoperbutylacetonicum* grown in glucose at the laboratory scale. Deviation bars are standard deviations based on average values from triplicate runs. This fundamental study allows us to determine the growth rate and show that biosolvents are produced as metabolites during exponential growth. These data correlate with the figure above where once the pH approaches 5.0 due to acidogenesis, the organism switches to solventogenesis to generate ABE within 20 hours. Also once stationary phase ensues, the majority of solvents have been produced.

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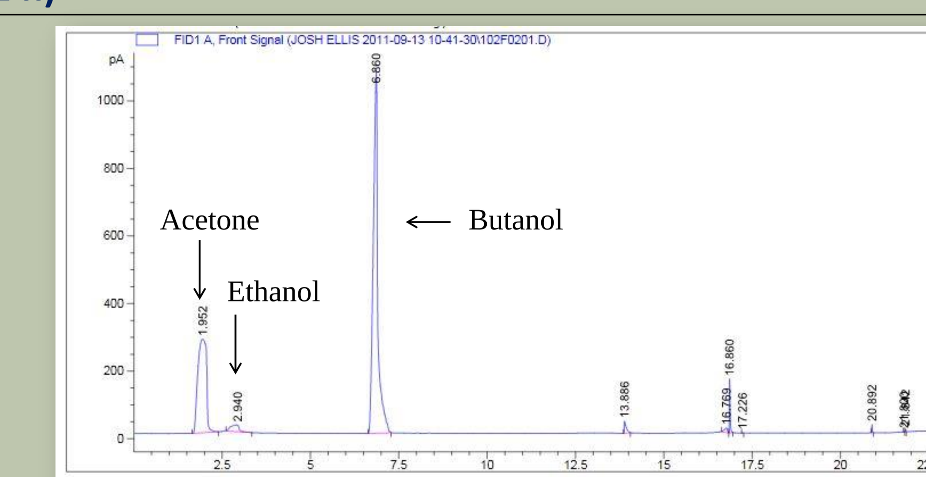
Solvent production by *C. saccharoperbutylacetonicum* N1-4 during batch fermentation using 10% pretreated algae at the laboratory scale. Measured yields are defined as grams of biosolvent produced per gram of sugar metabolized from algae biomass

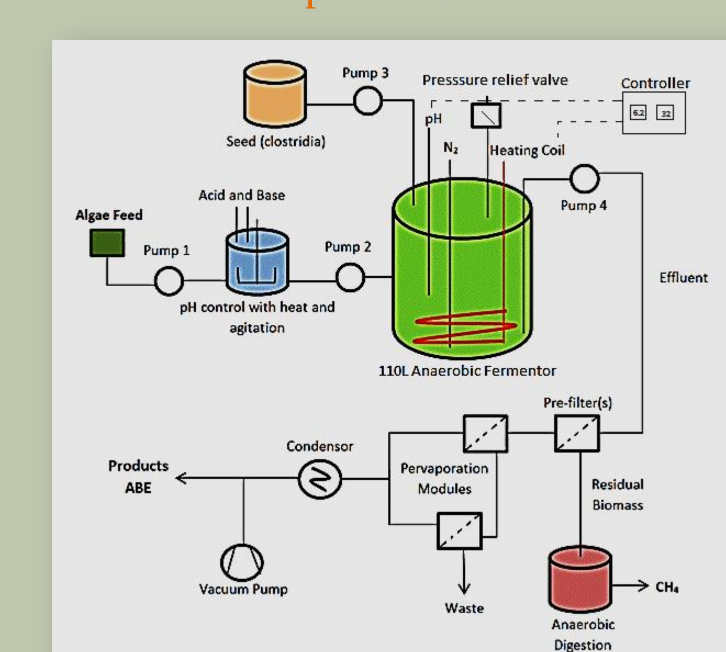
	Pretreated	Pretreated + 1% glucose	Pretreated + enzymes	Non-pretreated
Total ABE yield (g/g)	0.244	0.270	0.311	0.257
Butanol yield (g/g)	0.201	0.208	0.249	0.173
Acetone yield (g/g)	0.040	0.051	0.046	0.058
Total volumetric solvent productivity (g/L·h)	0.029	0.076	0.102	0.008
Volumetric butanol productivity (g/L·h)	0.020	0.059	0.081	0.005
Volumetric acetone productivity (g/L·h)	0.004	0.014	0.015	0.002



GC chromatogram quantifying ABE produced in the 110L fermentor

Conclusions, Significance, and Future Work

- A total of 9.89 g/L of overall ABE was produced at 68 hours of fermentation at the pilot scale.
- Acetone, butanol, and ethanol production from wastewater microalgae has been demonstrated at the laboratory scale using *C. saccharoperbutylacetonicum*.
- These bioproducts are both high value as well as renewable energy compounds.
- Measured yields and productivities at the laboratory scale are comparable to other published literature when different substrates were used (ex: corn fiber arabinoxylan, starch, glucose).
- Successful production of ABE from wastewater microalgae at the pilot scale will stimulate both the production of clean and renewable energy while stimulating national energy economies.
- Currently, pilot scale fermentations are underway using microalgae in the 110L bioreactor.
- Our objective is to make ABE from wastewater algae a commercially available and affordable bioproduct.



ABE production and purification schematic showing the process for scaling up ABE fermentation along with purifying biosolvents using a series of pre-filters and selective hydrophobic membranes. Once successful production of ABE from wastewater microalgae is achieved, our research group will work to provide purified biosolvents for further analyses.

References

- **Ellis, J.T.,** Miller, C.D., Sims, R.C. 2011. Methods for Producing Acetone, Butanol, and Ethanol from Algae. Patent Pending. United States Patent Application no. 13/663,002 and Patent Cooperation Treaty (PCT) Application no. US2012/062444
- **Ellis, J.T.,** Hengge, N., Sims, R.C., Miller, C.D. 2012. Acetone, butanol, and ethanol production from wastewater algae. *Bioresource Technology* 111, 491-495